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(54) METHOD FOR DETECTING THE FERTILE PERIOD

(71) We, WESTON LABORATORIES INC., a corporation of the State of Illinois, United States of America, of Hitt and Swanson Streets, Ottawa, Illinois, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a novel analytical test implement useful in detecting an increase in certain enzyme activity which is coincident with the fertile period of the female, and to a novel method for detecting the said increase in enzyme activity and thereby the fertile period.

There has been much interest in the past as well as presently, in being able to predict and detect the period of time during which the female is fertile, i.e., is capable of conceiving an offspring. The fertile period occurs during ovulation, when the ovum has departed the ovary and is still alive and in a position to be fertilized.

The importance is readily apparent of being able to predict and detect the fertile period, both to those who wish to take advantage of the fertile period and those who wish to avoid it. However, prior to the present invention, and methods and means available for such prediction and detection have failed to provide the degree of convenience and accuracy desired. Attempts to calculate said period by reference to the onset of menses are speculative and beset with inaccuracy because the human female, for instance, may ovulate at varying and unpredictable times. On the other hand, mechanical methods such as by taking and recording temperatures, while possibly more accurate, are distinctly inconvenient and may require considerable skill in observing and interpreting the data.

It has been found that certain enzymes found in the saliva, or other body secretions, (e.g., cervical mucous) vary in concentration during the first part of the female's fertile period and at the time of ovulation. Of particular interest among these enzymes are alkaline phosphatase, monosterase and acid phosphatase.

In our copending application No. 53901/67 (Serial No. 1203620) there is described a test implement useful in detecting an increase in alkaline phosphatase activity which is coincident with the fertile period of the female, which comprises a bibulous material impregnated with a non-toxic buffer capable of maintaining the pH at a level in the range of 10.0 to 10.3 and an indicator selected from indoxyl phosphate, five - bromo - indoxyl phosphate, 3 - o - methyl fluroescein phosphate, phenolphthalein mono-phosphate, and phenophthalein diphosphate, which indicator in the presence of said increased alkaline phosphatase activity forms a coloured compound.

In accordance with the present invention however there is now provided a test implement useful in detecting an increase in monoesterase activity which is coincident with the fertile period of the female, which comprises a bibulous material impregnated with a non-toxic buffer capable of maintaining the pH at a level in the range of 7.0 to 9.0 and an indicator selected from indoxyl acetate five - bromo - indoxyl acetate, 3 - o methyl fluorescein acetate, and phenolphthalein acetate which in the presence of said increased monoesterase activity forms a coloured compound.

The present invention also provides a novel method for detecting the fertile period of the female by indicating an increase in monoesterase activity in saliva or other body fluid which comprises contacting a sample of saliva or other body fluid from a female with the test implement defined in the preceding paragraph. The test implement (for example test paper) will change color during the period of ovulation and fertility of the female while it will undergo no color change at other times.

The cleavage of the ester bond in the aforesaid indoxyl compounds releases the indoxyl radical, or substituted indoxyl radical and, in the presence of oxygen or air two indoxyl

[*Price 5s. 0d. (25p)*]

radicals combine to form indigo, or a substituted indigo, a dark blue dye. This reaction normally takes place in a short period of time, such as 30 seconds to 4 minutes. The degree of blue formed is relative to the amount of the monoesterase present.

When phenolphthalein acetate is used, cleavage of the ester bond frees a phenolphthalein radical which will turn to a dull red color when the test implement is dipped into a solution having a pH in the range of about 8.3 to 10.0. Cleavage of the fluorescein ester releases substituted fluorescein which is yellow. The degree of the discernible color developed is principally dependent upon the amount of the monoesterase present.

The great advantage and convenience of this oral test is at one apparent. The female simply touches the test paper to her tongue to wet it, and waits a few minutes to see if a color change develops on the test paper. The result is easily observed, and does not require the recording and interpretation of data over a long period of time. Each test is complete per se. The test is reliable and simple as well as being convenient and relatively inexpensive.

Describing in more detail the test implement the implement is preferably made from bibulous paper, for example, an absorbent and chemically pure grade of filter paper or the like. Cloth strips of porous and absorbent wood strips may also be employed. The test paper is impregnated with a solution of the active ingredients and is then subjected to drying to evaporate the solvents and leave the active ingredients on the paper.

In one representative formula, when indoxyl acetate is the indicator, 6.9 grams of Methocel, a methyl cellulose product, is dissolved in 440 cc. distilled water, and 100 cc. of ethyl alcohol and 250 cc. propyl alcohol are added. Four grams of sodium citrate and 70 milligrams of citric acid are dissolved in 60 cc. of water. 1.5 grams of indoxyl acetate is dissolved in 100 cc. of ethyl alcohol with 12.5 grams of Tween 80 (a Registered Trade Mark denoting a polyoxyethylene derivative of sorbitan).

The three solutions are combined with the resultant solution having a pH of about 7.6. The large sheets of chemically pure filter paper are impregnated with the mixed solution and air dried.

When five - bromo - indoxyl acetate, phenolphthalein acetate or 3 - o - methyl fluorescein acetate is substituted in the above representative formulation the formulation remains the same, except the amount of the indicator used will vary from 50 milligrams to 2500 milligrams depending on the degree of final color desired.

The test papers or test tapes so prepared have been used in clinical studies to determine their accuracy in detecting the fertility period as evidenced by changes in the saliva of the female. When compared to the previous standard, namely, the (basal body temperature) thermal shift response, the foregoing test tapes give a significant correlation.

There is thus provided a convenient and accurate test method and test paper for detecting the fertile period by a simple test on saliva. The test papers above described, after contact with saliva during the fertile period, will detect an increase in monoesterase activity by developing a readily discernible color after but a short period of time. If there has been, no significant increase in monoesterase activity, the tape does not develop the readily discernible color upon contact with saliva.

While the foregoing test method and test tape are obviously of primary importance to the human female, it is apparent that the test is applicable to other female animals, and can be of considerable importance.

The response of the test implement may also be demonstrated by contacting other closely related body fluid, e.g. cervical mucous, with the test implement.

We make no claim herein to diagnostic tests carried out on human body fluid. Subject to the foregoing disclaimer.

WHAT WE CLAIM IS:—

A test implement useful in detecting an increase in monoesterase activity which is coincident with the fertile period of the female, which comprises a bibulous material impregnated with a non-toxic buffer capable of maintaining the pH at a level in the range of 7.0 to 9.0 and an indicator selected from indoxyl acetate, five bromo-indoxyl acetate, 3-o-methyl fluorescein acetate, and phenolphthalein acetate which in the presence of said increased monoesterase activity forms a coloured compound.

2. A test implement of claim 1 wherein said indicator is indoxyl acetate.

3. The test implement of claim 1 where said indicator is five bromo-indoxyl acetate.

4. The test implement of claim 1 wherein said indicator is 3-o methyl fluorescein acetate.

5. The test implement of claim 1 wherein said indicator is phenolphthalein acetate.

6. The method for an increase in monoesterase activity in saliva or other body fluid which is coincident with the fertile period of the female which comprises contacting the saliva or other body fluid with a test implement according to claim 1.

7. The method of claim 6 wherein said indicator is indoxyl acetate.

8. The method of claim 6 wherein said indicator is five bromo-indoxyl acetate.

9. The method of claim 6 wherein said indicator is 3-o methyl fluorescein acetate.

10. The method of claim 6 wherein said indicator is phenolphthalein acetate.

11. A test implement useful in detecting

an increase in monoesterase activity, substantially as hereinbefore described.

12. A method of detecting an increase in monoesterase activity in saliva or other body fluid substantially as hereinbefore described.

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